NUTRITIONAL STUDIES OF THE DERMATOPHYTES WITH SPECIAL REFERENCE TO *TRICHOPHYTON MEGNINI* BLANCHARD 1896 AND *TRICHOPHYTON GALLINAE* (MEGNIN 1881) *COMB. NOV.**

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As is well known, three genera of Dermatophytes are recognized on the basis of microscopic morphology in culture; namely, *Trichophyton*, *Microsporum* and *Epidermophyton*. Differentiation of species is not a great problem in the last two genera since they are few in number and sufficiently characteristic to make identification relatively simple. In the genus *Trichophyton*, however, a different situation exists. A large number of species has been described for this genus since the time of the original description of *Trichophyton tonsurans* by Malmsten in 1845 (1). At the time Sabouraud published "Les Teignes" in 1910 (2), he listed 31 species in the genus *Trichophyton*, basing species differentiation on the morphology of the fungus colony on a peptone-carbohydrate medium, his so-called "*Milieux d'epreuve*." From time to time it has been shown that many of these were not distinct species, but merely variants or mutations of one or another of the well-recognized types. There is still some disagreement among both European and American mycologists as to the number of species they recognize, and also which ones should be regarded as variants and which should fall into synonymy.

The increased interest in the nutrition of microorganisms and its application to the study of the dermatophytes has been a great aid in recognizing relations between these forms. For some time now this Department has been engaged in studies of this sort. In one instance this resulted in a reduction in the number of species (3). In another it has added criteria for the differentiation of species (4). Certain media favor the production of microscopic structures or spores for one species, while other ingredients may be needed by other species for the same purpose (5, 6, 7). Others who contributed much of value to this problem are Robbins and Ma (8), Lewis and Hopper (9), Giblett and Henry (10), and Mosher et al. (11), McVeigh and Campbell (12), Burkholder and Moyer (13).

These studies have shown the dermatophytes to be heterotrophic organisms, some of them requiring complex organic substances such as vitamins, carbohydrates and amino acids, not only for growth, but also for the production of characteristic colony patterns, pigments, spores and other microscopic structures which constitute taxonomic criteria for the identification of these organisms. Further studies of this type particularly with the less familiar or questionable species of dermatophytes were indicated.

Tricophyton Megnini Blanchard and *Tricophyton gallinae* (Megnin) are two such organisms. They are included in the present study together with *Trichophyton rubrum*, and several varieties of *Trichophyton mentagrophytes*.

Trichophyton Megnini and Trichophyton gallinae.—The situation existing in regard to *Trichophyton Megnini* and *Trichophyton gallinae* may best be explained by a review of the literature. From the very beginning these two organisms have been consistently confused. The first to be described was *Trichophyton gallinae*. This fungus was isolated by Megnin from chicken favus. He reported his findings in 1881 (14) naming it *Epidermophyton gallinae*. In 1890 (15) he presented cultures of the organism before the Société de Biologie de Paris and in his discussion stressed the ability of this fungus to produce a diffusible pigment in culture which would stain the entire substrate a deep currant red. This is indeed a very characteristic and unique property of *T. gallinae*. *T. Megnini* on the other hand produces a

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This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service, Bethesda, Maryland.

Received for publication October 4, 1951.

deeper rose or maroon pigment on the reverse of the colony which does not extend beyond the outer limits of the submerged mycelium. Notwithstanding this difference, Sabouraud in 1894 (16), when first confronted with a culture of *T. gallinae* from a case of chicken favus, considered it identical with the "*Trichophyton à culture rose*" he himself had previously described from tinea barbae in 1893 (17). In 1896 Blanchard (18) in his chapter on "Vegetable Parasites" in Bouchard's "*Traité de Pathologie Exotique*," included a description of the two species, giving the name *Trichophyton Megnini* to the organism which Sabouraud had isolated from ringworm of the beard, and retaining the name *Epidermophyton gallinae* for Megnin's organism from chicken favus. Blanchard's description of *T. Magnini* leaves no doubt that he is discussing Sabouraud's culture. He states, however, that both Megnin and Sabouraud studied the fowl disease and that the latter considered the cultures to be the same. Nevertheless, the fact remains that Blanchard himself described them separately. Sabouraud (2) corrected his error in a later publication, where, presenting data from comparative studies, he stated the cultures were distinct. In addition Muijs in 1916 (19) called attention to the difference in diffusibility of the red pigment produced by the two organisms in culture. The name *T. Megnini* Blanchard 1896 is therefore the first valid name given to the rose-colored organism described by Sabouraud from tinea barbae. It is in accordance with the Rules of Botanical Nomenclature and has priority over the others. Sabouraud's descriptive phrase "à culture rose" is not valid. All other binomials applied to this fungus should fall into synonymy as follows:

Trichophyton Megnini Blanchard 1896. Syn: *Trichophyton à culture rose* Sabouraud, 1893; *Trichophyton roseum* Bodin 1902; *Trichophyton rosaceum* Sabouraud, 1909; *Ectotrichophyton Megnini* Castellani and Chalmers, 1919; *Megatrichophyton Megnini* Neveau Lemaire, 1921; *Aleurosporia rosacea* Grigorakis, 1925; *Trichophyton* (*Aleurosporia*) *rosaceum* Guiart and Grigorakis, 1928; *Megatrichophyton roseum* Dodge, 1935.

In the case of *Epidermophyton gallinae*, credit for the species should go to Megnin (14). The morphologic characters of this organism, however, are incompatible with the diagnostic criteria recognized for the genus *Epidermophyton*. For this reason the organism has been placed in a variety of genera including *Achorion* and *Sabouraudites*, thus increasing the confusion which has continued to the present day.

Current textbooks on medical mycology and dermatology continue to present the two organisms with a certain degree of obscurity, suggesting, either that *T. Megnini* may be the same as *E. gallinae* or that *T. Megnini* may be a more deeply pigmented form of *Trichophyton rubrum*. Our present study has given us evidence that these two organisms are sufficiently different to deserve recognition as two distinct species. We believe the correct name for Megnin's organism to be *Trichophyton gallinae* with the following synonymy:

Trichophyton gallinae (Megnin) 1881, comb. nov.; *Epidermophyton gallinae* Megnin, 1881; *Lophophyton gallinae* Matruchot and Dassonville, 1899; *Achorion gallinae* Sabouraud, 1910; *Sabouraudites* (*Aleurocloster*) *gallinae* Ota and Langeron 1923; *Closteroaleurosporia gallinae* Grigorakis, 1925; *Microsporum* (*Closteroaleurosporia*) *gallinae* Grigorakis, 1929; *Sabouraudites gallinae* Langeron and Milochevitch, 1930.

MATERIALS AND METHODS

Organisms studied

C-1 — *Trichophyton rubrum*—isolated in Vanderbilt Clinic, Columbia-Presbyterian Medical Center, 1949

C-2 — *Trichophyton mentagrophytes*—"red-pigment-producer," isolated by Dr. Lucille Georg, Chamblee, Georgia, 1949

C-6, C-7, C-8	*Trichophyton rubrum*, isolated in Dr. Carrión's laboratory, Puerto Rico, 1946, 1947, 1948
C-9	*Trichophyton lacticolor*, isolate brought by Dr. J. Gardner Hopkins from Dr. Sabouraud's laboratory in Paris, 1948
C-10	*Trichophyton farinulentum* (same source as C-9)
C-11	*Trichophyton rosaceum* (same source as C-9, strain Gaillard)
C-12	*Trichophyton rubrum*—isolated in Vanderbilt Clinic, Columbia-Presbyterian Medical Center, 1950
C-13 and C-14	*Trichophyton mentagrophytes*, Vanderbilt Clinic, 1950
C-15	*Sabouraudites gallinae* (same source as C-9)
C-16	*Trichophyton rosaceum*—isolate from C.B.S., Holland, courtesy of Dr. Elizabeth Hazen, 1950
C-20, C-21, C-22, C-23	*Trichophyton rosaceum*, isolates obtained in 1950 from Dr. Rivalier, Hospital St. Louis, Paris—strains Baticle, Leendert, Oscar and "W" respectively
C-24	*Sabouraudites gallinae*—isolate obtained in 1950 from Dr. Rivalier—strain "P"

Media

Studies were made on various complex media commonly used by mycologists in the study of the dermatophytes and on chemically defined media as listed below.

A. Complex media. 1. Sabouraud's Dextrose Agar (Difco), 2. Sabouraud's Honey Agar, 3. Potato Dextrose Agar (Difco), 4. Wort Agar (Difco), 5. Corn Meal Infusion Agar, 6. Corn Meal Infusion Agar plus dextrose, 7. Bacto Blood Agar Base (Difco).

The Sabouraud's Honey Agar and the Corn Meal Infusion Agar were prepared in this laboratory according to the following formulas.

Honey agar

(1) Honey	60 g.	Heat in Arnold sterilizer to dissolve; filter through paper
Peptone (Fairchilds)	10 g.	
Water	500 cc.	
(2) Agar	20 g.	Heat to dissolve; add to (1), adjust pH to 5.5, tube, and autoclave 25 minutes at 15 lbs. pressure.
Water	500 cc.	

Corn-meal Agar

Corn-meal Water	62.5 g. 1500 cc.	Heat at 60 C for 1 hour, filter through paper and make up to 1500 cc. Add 19 g. agar, heat in Arnold sterilizer for 1½ hour, filter through cotton and tube. Sterilize in the autoclave for 25 minutes at 15 lbs. pressure. pH as made up is about 6.8 to 7

Corn-meal Agar plus dextrose

One per cent Dextrose C.P. was added to the above formula

B. Chemically defined media. A "basal" medium was prepared with the following formula:

$MgSO_4 \cdot 7H_2O$	0.1	g. per liter
Dextrose, C.P.	40.	g. per liter
Sorensen's M/15 phosphate buffer mixture to give a final pH of 7.0.	100	ml. per liter
"Purified" agar	20.	g. per liter
Distilled water to make	800	ml.

To this "basal" medium nitrogen sources and vitamins were added individually or in various combinations. They were dissolved separately in 200 ml. distilled water and added to the media in the following concentrations:

MEDIUM CODE NUMBER	ADDITIONS
N-1	0.2 per cent ammonium chloride
N-2	0.2 per cent ammonium nitrate
N-3	0.2 per cent asparagine
N-4	0.1 per cent casein (G.B.I. vitamin-free casein hydrolysate)
N-5	0.01 per cent *dl*-alpha-alanine
N-6	0.01 per cent 1(+) arginine monohydrochloride
N-7	0.01 per cent glycine
N-8	0.01 per cent *dl*-valine
N-9	0.0172 per cent *dl*-alpha-alanine 0.0097 per cent 1(+) arginine 0.013 per cent glycine 0.02 per cent *dl*-valine 0.014 per cent asparagine
N-10	0.0589 per cent 1(+) histidine
N-11	0.1205 per cent 1(−) leucine
N-12	0.0843 per cent 1(+) lysine
N-13	0.1066 per cent 1(−) proline
N-14	0.0147 per cent 1(+) histidine 0.0301 per cent 1(−) leucine 0.021 per cent 1(+) lysine 0.0266 per cent 1(−) proline

The powdered Difco Agar was purified by washing and treating with pyridine. The residue was then extracted with alcohol and acetone and dried before use. The asparagine was treated with norit-A and recrystallized from alcohol.

Methods

For studies on the usual complex media, cultures were made in Petri dishes and observed at the end of one, two, and six weeks. Slide cultures were also made on these same media.

For studies on chemically-defined media, agar slopes were employed. The ability of each isolate to utilize a given substrate as well as its behavior in it was observed and recorded. All cultures were maintained in an incubator at 27 C. Slide cultures and modified "growth tubes" were also used.*

EXPERIMENTAL

Studies on complex media

Observations were made of the cultures on the usual media in order to observe the range of variation in colony pattern, pigment production and microscopic morphology. This proved very fruitful in the study of *Trichophyton Megnini* and *Trichophyton gallinae*, as it resulted in the confirmation of the publications of Blanchard (1896) (18), Sabouraud (1910) (2), and Muijs (1916) (19), who pointed out the differential characteristics of these two organisms. These observations are briefly summarized in Table I.

The predominant microscopic structure of *T. gallinae* is the macroconidium which may explain the placing of this fungus in the genus *Sabouraudites* (= *Microsporum*) by Ota and Langeron (1923) (20) and Langeron (1945) (21). This macroconidium, however, is characteristic of the genus *Trichophyton*, being thin-walled and fusiform. The production of microconidia by this organism would also exclude it from the genus *Epidermophyton*. We therefore recommend that the new combination *Trichophyton gallinae* (Megnin 1881) be used for this organism.

From these observations the value of the complex media are summarized:

1. Sabouraud's dextrose and Sabouraud's honey agar serve adequately for colony characteristics and pigment production for each of the species studied. (Figs. 1 and 2)

2. The media most adequate for the production of macro- and microconidia vary with the species. For *T. mentagrophytes*, wort agar is satisfactory, as is corn meal agar and heart infusion tryptose agar. This last is especially favorable for production of macroconidia of *T. rubrum* which are rarely produced on any of the other media. *T. Megnini* produces microconidia in great abundance on the complex media. They are long, clavate to pyriform in shape, the size ranging

* The "tube method" technic was described by Ryan, Beadle and Tatum in 1943 (23) for daily measurements of the rate of growth of Neurospora. The modification of this method used by Silva in her studies on the physiology of the fungi of chromoblastomycosis was employed. She found that due to the slow rate of growth of the pathogens, a short narrow test tube was more convenient than the glass tubing used by Ryan et al. The tube prevented too rapid drying of the agar. A dent was made on one side of the wall near the open end of the tube. This made it possible to keep the tube horizontal and not have the agar touch the cotton plug. Inoculations were made close to the mouth of the tube by drawing the needle across the surface of the agar in a line perpendicular to the long axis of the tube.

TABLE I

Characteristics of Trichophyton Megnini and Trichophyton gallinae on the usual complex mycological media

	T. MEGNINI	T. GALLINAE
	1. Macroscopic	
A. Sabouraud's dextrose agar	Colony somewhat raised, with velvety to powdery aerial mycelium; surface radially folded and occasionally cracked	Colony flat and discoid with chamois-like surface, radial folds and convolutions
	Aerial mycelium at first white, later becoming pink to deep rose	Aerial mycelium becomes tinted salmon buff to light pink
	Pigment in under surface brown, deep red or maroon, remains confined to area occupied by submerged mycelium	Diffusible pigment extends beyond the area occupied by the colony, tinting the entire substrate a bright strawberry red
B. Honey agar	Essentially as on Sabouraud's dextrose agar	
C. Potato dextrose agar	Colony consists chiefly of submerged mycelium, reddish brown or maroon in color	Colony consists of colorless aerial and submerged mycelium with soluble pigment staining large area of surrounding agar pale orange to buff
D. Corn meal infusion agar	Colony submerged and showing rose to red pigment	Colony submerged, opalescent white and producing no pigment
E. Blood agar base	Colony dry, powdery, radially folded with white to bluish-gray aerial mycelium; auburn under surface	Colony waxy-looking and honey-combed, ochraceous buff in color. No soluble pigment
	2. Microscopic	
	Microconidia abundant in all the usual media; long-clavate to pyriform (2.5 to 9.5 μ x 1.2 to 2.9 μ) sessile or pedunculated, firmly attached to long, unbranched conidiophores *en thyrse*	Microconidia scarce. When present, spherical to pyriform and attached *en thyrse*
	Macroconidia scarce but have been observed on Sabouraud's dextrose and honey agars, wort agar and B.A.B.; clavate to pencil-shaped, 1 to 5 celled; 12 to 47.2 μ long, 2.5 to 7.2 μ wide	Macroconidia abundant particularly on casein and yeast extract media; thin-walled and fusiform, 3 to 7-celled; 21 to 41 μ long, 4 to 8 μ wide

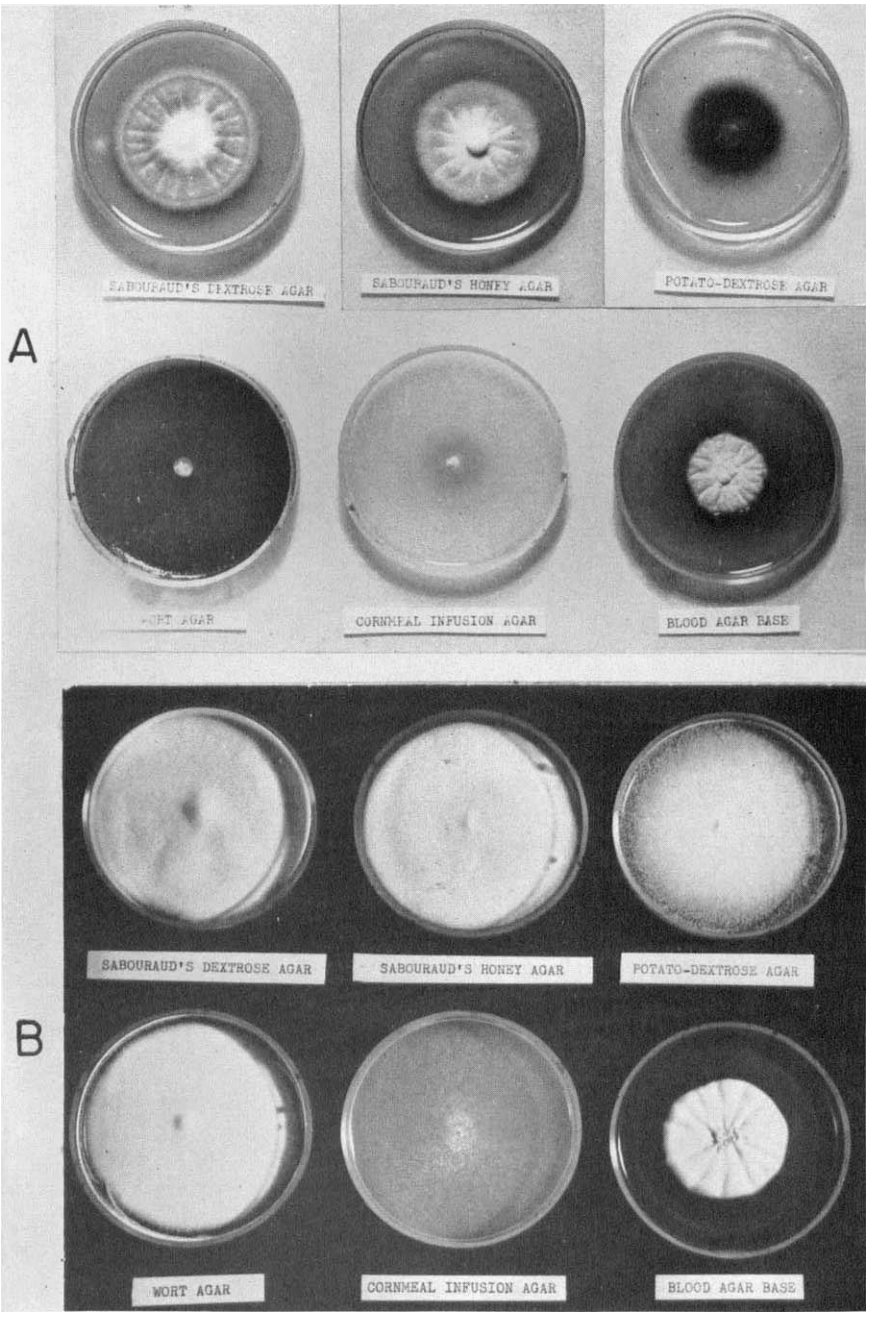

FIG. 1. Two week old colonies on the usual complex media. A, *Trichophyton rubrum* (strain C-12); B, *Trichophyton mentagrophytes* (strain C-2).

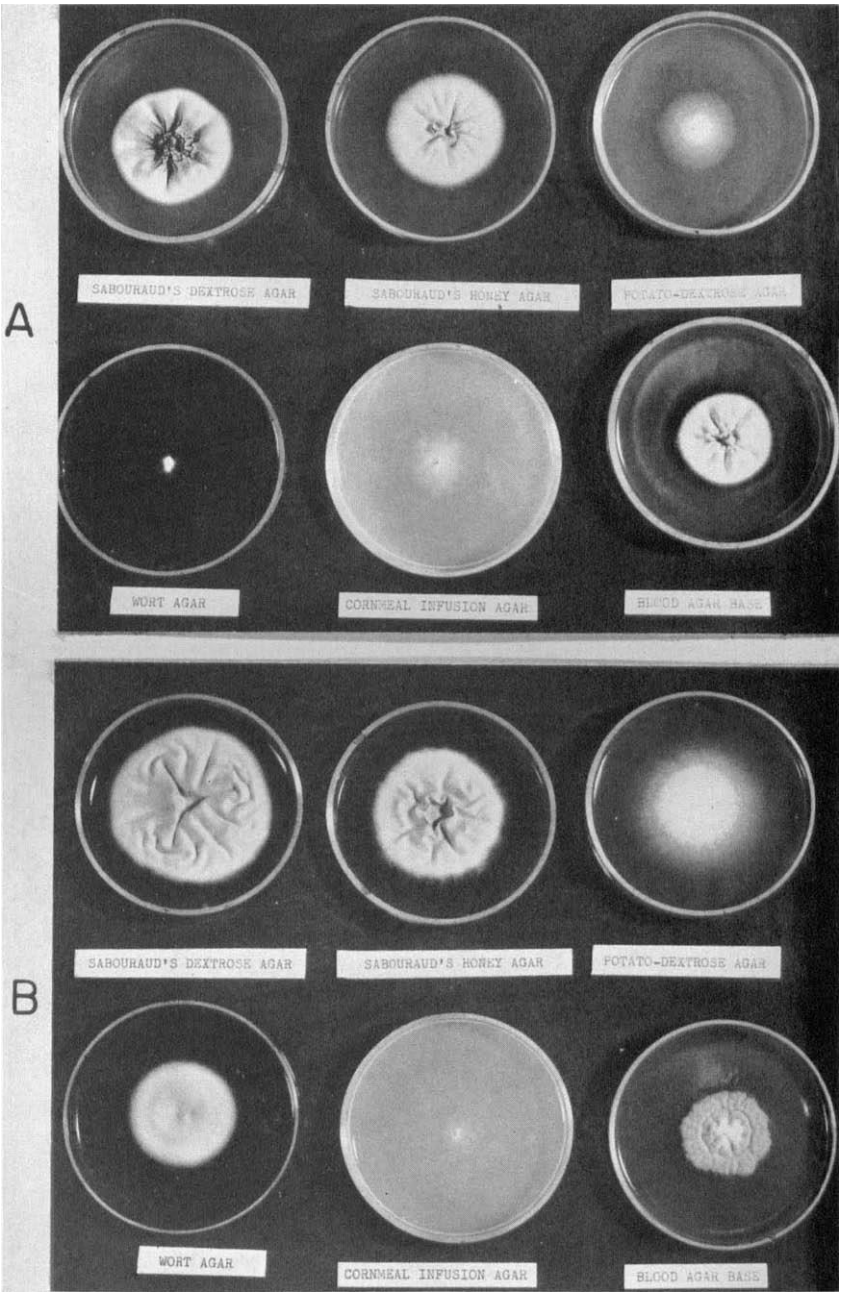

FIG. 2. Two week old colonies on the usual complex media. A, *Trichophyton Megnini* (strain C-23); B, *Trichophyton gallinae* (strain C-15).

from 2.5 to 9.5μ x 1.2 to 2.9μ. They may be sessile or attached to the unbranched conidiophore by a short stalk, i.e. "en thyrse." Macroconidia are scarce, clavate to pencil-shaped, 1–5 celled, the length 12 to 47.2μ and the width 2.5 to 7.2μ.

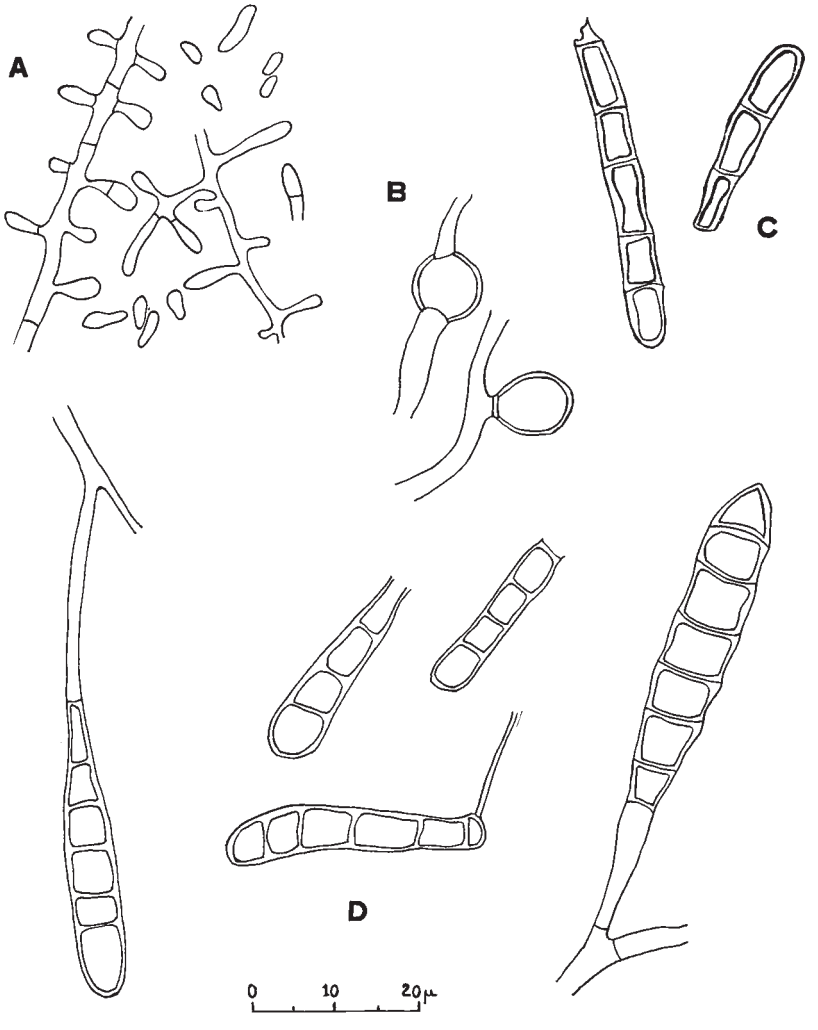

FIG. 3. Camera lucida drawings of A, microconidia, B, chlamydospores and C, macroconidia of *T. Megnini;* D, macroconidia of *T. gallinae.* A on yeast extract, B and D on casein hydrolysate, C on blood agar base.

In *T. gallinae,* it is the microconidia that are scarce. When found they are spherical to pyriform and borne "en thyrse". The macroconidia are abundant both on the casein hydrolysate medium and the yeast extract agar. They are thin-walled, fusiform, 3 to 7-celled, and measure 21 to 41μ by 2 to 8μ. They are characteristic of the genus *Trichophyton.* (Fig. 3D).

3. As may be seen from Plates 1 and 2 the growth of these organisms on potato-dextrose agar and on corn meal infusion agar is usually submerged with only a thin veil of aerial mycelium. The exceptions to this are *T. mentagrophytes* and *T. gallinae* both of which show an abundant growth of aerial mycelium on potato dextrose agar. The pigment produced by the submerged mycelium on these media is more easily differentiated than that produced on the richer media. It is only when dextrose is added to corn meal agar that *T. rubrum* will form pigment, whereas *T. mentagrophytes* rarely forms any pigment on corn meal even in the presence of dextrose. *T. Megnini* produces a rose pigment on corn meal agar with or without dextrose, whereas *T. gallinae* produces a pale apricot or buff on potato dextrose and either no pigment or a pale buff on corn meal with or without dextrose. The pigment production of these strains on the three media is summarized in Table II.

TABLE II

Pigment production of T. rubrum, T. mentagrophytes, T. Megnini and T. gallinae on potato dextrose agar and on corn meal infusion agar with and without dextrose

ORGANISM	MEDIUM		
	Potato Dextrose	Corn Meal Infusion	Corn Meal Infusion Plus Dextrose
T. rubrum	Intense wine-red	No pigment	Wine-red
T. mentagrophytes	Pale yellow to orange to red-brown	No pigment or pale rose to brown	No pigment or orange to yellow
T. Megnini	Deep red	Rose-red	Pink
T. gallinae	Apricot buff soluble	No pigment	No pigment or pale buff

Studies on chemically defined media

Comparison of inorganic and organic nitrogen sources.—Tests were made with four media (N-1 to N-4) containing respectively, ammonium chloride, ammonium nitrate, asparagine and casein hydrolysate as sole sources of nitrogen in order to compare their utilization in the presence or absence of vitamins.

Five ml. amounts were placed in each tube and slanted to give a one inch slope. Tiny fragments of inoculum, taken from a washed suspension made by grinding mycelium and spores from two-week old slant cultures in sterile distilled water were used. Duplicate cultures were made in each case. As controls each organism was planted on Sabouraud's dextrose agar and on the "basal medium" without nitrogen.

Results on the vitamin-free media.—There was no growth in the absence of nitrogen but all organisms grew well on the Sabouraud controls, showing that the inoculum was adequate. Of the 18 strains studied, 12 grew well on the four media whereas the remaining six grew only in the presence of 0.1 per cent casein (medium N-4). These six strains were all classified as *T. Megnini.* This would indicate that the 12 strains including five of *T. rubrum*, five of *T. mentagrophytes*

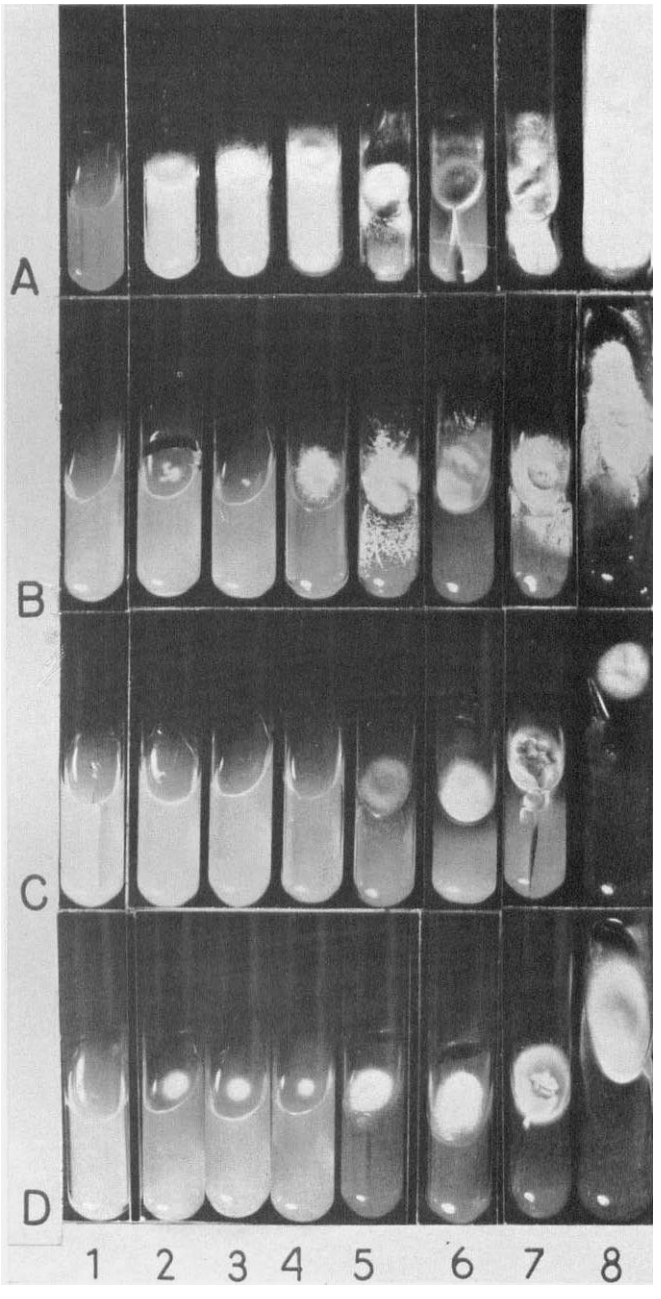

FIG. 4. A, *T. rubrum;* B, *T. mentagrophytes;* C, *T. Megnini;* D, *T. gallinae* on "basal" medium with various nitrogen sources. Tube 1, no nitrogen; 2, 0.2% NH_4Cl; 3, 0.2% NH_4NO_3; 4, 0.2% asparagine; 5, 0.1% casein hydrolysate; 6, 0.1% casein hydrolysate plus seven vitamins; 7, 1.0% casein hydrolysate; 8, Sabouraud's dextrose agar.

and two of *T. gallinae* were able to synthesize proteins not only from the single amino acid asparagine but also from the inorganic ammonium nitrogen. The growth of the four species on the above media is illustrated in Figure 4.

The result with the *T. mentagrophytes* strains including the varieties *farinulentum* and *lacticolor* is not in agreement with the findings of Robbins and Ma (1945) (8) for a single strain of *T. mentagrophytes*. They reported it unable to use ammonium nitrogen. Mosher et al. (1936) (11) had previously reported that *T. mentagrophytes* would not grow in a medium containing only inorganic nitrogen. McVeigh and Campbell have shown variation in the ability of *T. mentagrophytes* to utilize inorganic nitrogen. They used 6 variants of this species and found that they all grew on ammonium sulphate. They indicated, however, that the mycelial forms could utilize the nitrogen from this inorganic source and from simple organic sources more successfully than could the conidial forms. One of our strains, C-14, was a recently isolated, actively sporulating form, and its ability to utilize the ammonium nitrogen was equal to that of our other strains.

Since the media used were vitamin-free it may be concluded that all four species are autotrophic for the known vitamins. This observation had already been made for *T. mentagrophytes* (8, 11) and for *T. rubrum* (13), but not for *T. Megnini* and *T. gallinae*.

Results on media with added vitamins.—Seven vitamins were added to media N-1 to N-4 in the following concentrations per liter: 500 μg each of thiamine hydrochloride, riboflavine, pyridoxine hydrochloride, calcium pantothenate and niacin; 10,000 μg of inositol and 50 μg of biotin. In no instance was there noted any change in the amount or character of the growth from that produced on the vitamin-free media (Figure 4). The presence of the vitamins did not enable *T. Megnini* to utilize the inorganic nitrogen sources or asparagine.

Pigment production on these media, all of which contained dextrose, indicated that the nitrogen source is also of importance in this respect. The 5 strains of *T. rubrum* produced bright red pigment on both casein and asparagine, but only pale, or dark amber brown on the NH_4Cl and NH_4NO_3. The two strains of *T. mentagrophytes* (C-2 and C-10) labeled "red" strains, produced the red pigment on the casein medium only. *T. gallinae* produced a pale pink or neutral brown pigment on asparagine and casein, while *T. Megnini* produced a dark red pigment on the under surface of the colonies on casein.

These observations confirm the work of previous investigators in that:

(1) If a suitable carbohydrate is present, asparagine is a satisfactory source of nitrogen for pigment production by *T. rubrum* (9).

(2) Pigment production of *T. rubrum* will not take place in the presence of dextrose when the nitrogen source is NH_4Cl (4).

(3) Amino acids may influence the pigment production of *T. mentagrophytes* (8) and of *T. rubrum* (4).

Studies on amino acid media

Amino acids were added to the vitamin free "basal" medium to give various concentrations so as to test their effect on the growth and pigment production of the organisms under observation.

In one series of tests *dl*-alpha-alanine, *l*(+) arginine, glycine and *dl*-valine were added to the basal medium singly in quantities of 10 mg. each per 100 ml. of medium. (Medium N-5, N-6, N-7, and N-8). For determination of rate of growth these media were poured in 3 ml. amounts into modified growth tubes. Similar tubes were prepared with Sabouraud's media for controls. Three strains of *T. rubrum* (C-1, C-6, C-12); one each of *T. Megnini* (C-11); and *T. gallinae* (C-15) were studied in this manner.

Results on amino acid media.—The results at the end of 3 weeks are summarized in Table III. From this table it will be noted that *T. rubrum* and *T. gallinae*

TABLE III

Growth and pigment production of T. rubrum, T. Megnini and T. gallinae on the amino acids alanine, arginine, glycine and valine as the sole source of nitrogen

ORGANISM	MEDIUM									
	Alanine		Arginine		Glycine		Valine		Sabouraud's dextrose controls	
	Growth* in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment
T. rubrum										
C-1	15	None	1	None	13.5	None	6	None	40	Yellow
C-6	18	Pink	0		21	Pale rose	18	Pale rose	33	Maroon
C-12	16	Peach red	16	Peach red	20	Peach red	9	Peach red	24	Maroon
T. Megnini										
C-11	0		0		0		0		20	Blood red
T. gallinae										
C-15	9	None	20	None	12	Pink soluble	12	None	25	Maroon under colony; red diffusible

* Growth = diameter of colony at end of three weeks.

grew well on each of the four amino acids tested while *T. Megnini* failed to grow on any of them. There was good growth on the Sabouraud control tubes in each case, and the rate of growth on this medium was greater than on the individual acids.

In regard to pigment, two of the *T. rubrum* strains (C-6 and C-12) produced light to medium red pigment on each of the four amino acids whereas the third strain (C-1) failed to produce pigment on any of them. However it should be noted that strain C-1 produced only a weak pigment on Sabouraud's dextrose agar. In the case of *T. gallinae*, glycine alone of the four amino acids permitted pigment formation.

It had previously been shown that asparagine as well as these four amino acids failed to support growth of *T. Megnini*. To determine whether the amount of nitrogen available was adequate, tests were made with these amino acids in a nitrogen concentration that would approximate or equal that provided by a

0.1% casein hydrolysate (i.e. 12.9 mg. N per 100 ml. of media). Five media were prepared, each of which contained a single one of the amino acids in the above concentration. In addition medium N-9 was prepared, containing the five acids, combined so that each acid contributed 2.58 mg. N per 100 ml. of medium, the total concentration being 12.9 mg. N per 100 ml. medium.

In this series five isolates were tested, two of *T. Megnini* (C-11 and C-16), one each of *T. gallinae*, *T. rubrum* and *T. mentagrophytes* (C-15, C-6 and C-14). With this increased concentration of available nitrogen there was no significant

TABLE IV

Growth and pigment production of T. rubrum, T. mentagrophytes, T. gallinae and T. Megnini on the amino acids, histidine, leucine, lysine, and proline

ORGANISM	AMINO ACIDS									
	Histidine		Leucine		Lysine		Proline		Medium N-14	
	Growth* in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment
T. rubrum C-6	12	None	13	Wine-red to maroon	14	Wine-red to maroon	18	Wine-red to maroon	15	Wine-red, less intense
T. mentagrophytes C-14	22	Light to medium brown	23	Light to medium brown	20	Light brown	23	Light brown	20	Buff
T. gallinae C-15	8	None	7	None	15	Pale rose diffusible	13	None	20	Pale rose
T. Megnini C-11	8	Pale yellow to orange	0		0		0		15	Wine-red to maroon
T. Megnini C-16	4	None	0		0		0		13	Wine-red to maroon

* Growth = diameter of colony at end of three weeks.

variation in the amount or character of the growth or in the pigment produced by the strains tested. The only exception was *T. gallinae* which produced a diffusible rose pigment in asparagine, and a more intense and soluble pigment at the higher concentration of glycine.

The strain of *T. mentagrophytes* included in this study grew well on each of the five amino acids and on the combination of the same, producing granular to powdery aerial mycelium and a pale-orange to medium brown pigment on the under surface of the colony. The two *T. Megnini* strains failed to grow on any of the 5 amino acids, added singly or in combination.

A second group of amino acids, *l*(+) histidine, *l*(−) leucine, *l*(+) lysine, and *l*(−) proline, occurring in somewhat greater concentration in the casein hydrolysate than those of the previous group were tested with these same 5 isolates. The media were prepared with each of these acids in a concentration to give as in the above, 12.9 mg. N per 100 ml. In addition medium N-14 was prepared with these four amino acids combined so that each contributed 3.225 mg. N per 100 ml. of

TABLE V

Growth and pigment production of T. Megnini and T. gallinae on various combinations of histidine, leucine, lysine and proline

ORGANISM	MEDIUM N-14									
	Unmodified		Minus Histidine		Minus Leucine		Minus Lysine		Minus Proline	
	Growth* in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment	Growth in mm.	Pigment
T. Megnini										
C-11	13	Red to maroon	0		8	Rose	18	Red to maroon	5	Pale pink
C-16	13	Maroon	0		5		18	Red to maroon	13	Maroon
C-20	13	Deep red	0		10	Brown to red	18	Maroon	10	Brown to red
C-21	17	Maroon	0		17	Red to maroon	17	Maroon	15	Red to maroon
C-22	22	Dull red	0		17	Dull red	15	Red	18	Brown
T. gallinae										
C-24	13	Strawberry red diffusible	13	Pink diffusible	14	Pink diffusible	13	Yellow to orange diffusible	10	Deep pink diffusible
C-15	15	Deep pink diffusible	15	Pink diffusible	15	Red under surface; pink diffusible	10	Pale pink diffusible	12	Deep pink diffusible

* Growth = diameter of colony at the end of three weeks.

medium. The total N content was thus brought to 12.9 mg. N per 100 ml. of medium. Results of these studies are summarized in Table IV.

From this table the following facts are evident:

(1) *T. rubrum*, *T. mentagrophytes* and *T. gallinae* grew in each of the amino acids tested, and at comparable rates, whereas *T. Megnini* grew only in the presence of histidine.

(2) The pigment production of these organisms varied with the media; i.e., *T. rubrum* produced intense wine red pigments on leucine, lysine and proline

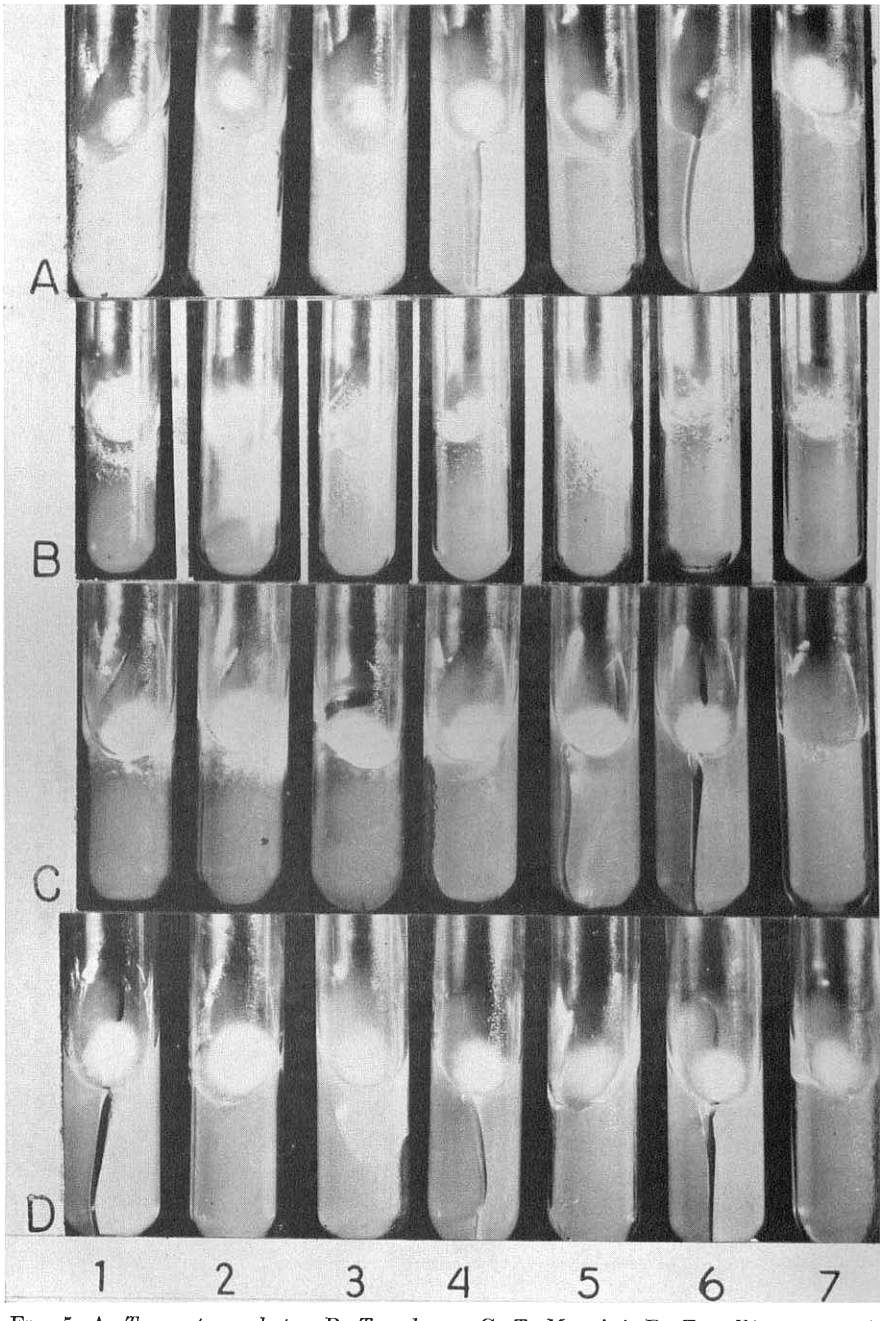

FIG. 5. A, *T. mentagrophytes;* B, *T. rubrum;* C, *T. Megnini;* D, *T. gallinae* on various modifications of medium N-14. Tube 1, N-14; 2, double the concentration of amino acids; 3, histidine increased 10 times; 4, lysine increased 10 times; 5, both histidine and lysine increased 5 times; 6, histidine increased 10 times, lysine omitted; 7, lysine increased 10 times, histidine omitted.

alone but none on histidine, and in media N-14 where the four amino acids were combined the intensity of the pigment was reduced. *T. mentagrophytes* on the other hand produced a light to medium brown pigment on the under surface of colony in each of the five media. *T. gallinae* produced a pale salmon-orange pigment on lysine but no pigment on the other three amino acids. The pigment occurred on the under surface of the colony and diffused through the medium. When the four acids were combined there was likewise a pale pigment on the under surface of the colony but it was not diffusible. *T. Megnini* produced a scant growth and a pale yellow to orange pigment on histidine; luxuriant growth and an intense red pigment when this amino acid was combined with the other three. The fact that *T. Megnini* did not grow in the absence of histidine seems to indicate that this amino acid is essential for the growth of this dermatophyte. This is another instance where a dermatophyte has been shown to have a specific amino acid requirement. Georg found this to be true of one strain out of eleven of *T. violaceum* studied (7) and for *T. Megnini* (22).

It seemed of interest because of the above facts to study further the amino acid requirements of *T. Megnini*. For this purpose, modifications of N-14 medium were prepared by omitting one at a time each of the amino acids. Five *T. Megnini* strains and two *T. gallinae* were tested. The results are summarized in Table V.

It will be noted that the *T. gallinae* strains grew well on all the media, but without lysine, pigment production was decreased. *T. Megnini* failed to grow in the absence of histidine even when the other three amino acids were present. In the absence of lysine *T. Megnini* showed an increased rate of growth, suggesting that this amino acid might have an inhibitory effect on the growth of this organism. As a result of this finding it seemed wise to study the effect of varying quantities of lysine and histidine, alone, and in combination. To the "basal" media histidine was added in concentrations of 0.01%, 0.05% and 0.10%, lysine in the same concentrations, and combinations of *l*(+) histidine and *l*(+) lysine as follows:

0.01% histidine and 0.01% lysine
0.01% histidine and 0.10% lysine
0.10% histidine and 0.01% lysine
0.10% histidine and 0.10% lysine

Five *T. Megnini* strains were tested with the following results:

(1) The amount of growth and the extent and intensity of the under surface pigment increased with increasing amounts of histidine.

(2) Lysine alone did not support growth in any of the three concentrations, when it was the sole source of nitrogen.

(3) Lysine in the presence of histidine did not inhibit growth in the proportions used. On the other hand increasing amounts of lysine in the presence of given amounts of histidine enhanced growth. It seems, therefore, that the presence of histidine enabled the fungus to utilize increasing amounts of lysine.

From this it seemed necessary to evaluate the amino acids lysine and histidine

in their relation to leucine and proline, the other amino acids contained in medium N-14. The following modifications of this medium were prepared.

(1) Concentration of the four amino acids doubled.
(2) Concentration of histidine increased 10 times.
(3) Concentration of lysine increased 10 times.
(4) Concentration of histidine and lysine each increased 5 times.
(5) Lysine omitted and histidine increased 10 times.
(6) Histidine omitted and lysine increased 10 times.

Five *T. Megnini* isolates (C-11, 16, 20, 21 and 22); two *T. gallinae* (C-15 and C-24) and one each of *T. mentagrophytes* (C-14) and *T. rubrum* (C-6) were tested on these six media, and in addition on medium N-14 as a control.

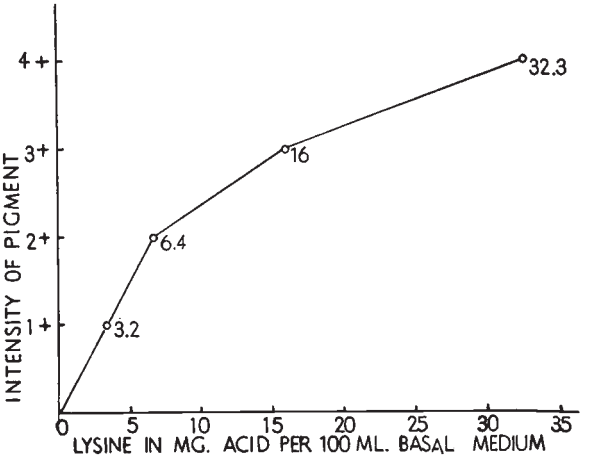

FIG. 6. To show the effect of the concentration of lysine on the intensity of the pigment produced by *T. gallinae* (C-24) (1+ = pale rose, 2+ medium rose, 3+ light red, 4+ medium red).

The results of these tests are illustrated in Figure 5 and may be summarized as follows:

(1) *T. mentagrophytes* and *T. rubrum* and *T. gallinae* grew equally well on all six media and at a rate comparable to that on N-14.

(2) *T. Megnini* failed to grow in the absence of histidine but grew at comparable rates on all the other media.

(3) The concentration of the amino acids did not visibly affect the pigment production of *T. rubrum*, *T. mentagrophytes* or *T. Megnini*, whereas a direct relationship was noted between the concentration of lysine in the medium and the amount and intensity of the diffusible pigment produced by *T. gallinae*, as illustrated in Figure 6.

SUMMARY AND CONCLUSIONS

Studies comparing the nutritional requirements of four species of *Trichophyton*, namely: *mentagrophytes*, *rubrum*, *Megnini* and *gallinae* are reported here.

The work of previous investigators showing that *T. rubrum* and *T. mentagrophytes* are autotrophic for the known vitamins has been confirmed. In addition this has been shown to be true also for *T. Megnini* and *T. gallinae*. Further, it was demonstrated that the addition of vitamins did not alter the ability of any of the four species to utilize the various nitrogen sources or change the rate of growth. Confirmation has been obtained that *T. rubrum* and *T. mentagrophytes* can synthesize proteins from inorganic ammonium nitrogen. This is also true for *T. gallinae*, but *T. Megnini* on the other hand requires histidine for this purpose.

Additional evidence of the importance of the nitrogen source in pigment production by these fungi has been disclosed. The presence and concentration of lysine seems to determine the amount and intensity of the diffusible pigment produced by *T. gallinae*.

Evidence is presented from a review of the literature and from our studies to show that *T. Megnini* and *T. gallinae* are distinct species.

It is concluded from a review of the literature that *T. Megnini* is the valid name for this species. A list of the synonyms is given.

A new combination *Trichophyton gallinae* is proposed for the *Epidermophyton gallinae* of Megnin and a list of synonyms given.

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